SUMMARY

In the **Introduction** the principal methods for the assessment of the extent and rate of bioavailability are reviewed, and their limitations and drawbacks are shortly discussed. Since a direct numerical deconvolution technique with staircase approximation seemed to meet the requisites of the 'ideal method' rather well, this method was chosen as a starting point for the development and validation of an appropriate method for the determination of drug input.

In **Chapter I** the theoretical basis of the application of linear time-invariant systems is presented. The interpretation of the term 'drug input' depends on the route of administration of the reference dose, also referred to as unit dose impulse input. The term 'drug input' may be defined as the appearance of unchanged drug in the general circulation (definition of bioavailability) in the case where the unit dose impulse input is an intravenous bolus administration. Alternatively, if the unit dose impulse input is an orally administered solution of the drug, 'drug input' is the appearance of dissolved drug in the gastro-intestinal fluids, or the in vivo drug release from a solid oral dosage form.
A numerical deconvolution algorithm is derived by approximating the input rates during the intervals between data points by constants. This staircase approximation algorithm requires the estimation of integrals (partial areas under the curve) of the unit dose impulse response function.
It is demonstrated that, if the unit dose is an intravenous bolus injection in a multi-compartment model (abbreviated to m-CBM/iv system), the integrals may be obtained accurately by simple numerical methods, e.g., the logarithmic trapezoidal rule and Simpson's rule, in combination with interpolation and extrapolation methods. The effects of the pharmacokinetic parameters of the system and the time schedule for sampling are evaluated. It is not necessary that the data points are equally spaced. However, for practical reasons equal intervals between the data points in the early phase, which may be doubled every three or four data points, are recommended. In contrast with the AUC ratio method, statistical moments, and curve-fitting methods, it is not necessary to sample after the absorption phase. As a major advantage over the Loo-Riegelman method and the deconvolution method proposed by Vaughan, curve-fitting of the intravenous data is avoided.
A new method, using a constant input rate of the reference dose instead of an impulse input, is proposed. This constant input method seems to be superior to the usual methods, since the integrals can be determined exactly for any unit dose impulse response function. In particular, the constant input method avoids estimation of the unknown response profile immediately after intravenous bolus administration. Moreover, the method is also applicable for drugs which cannot be administered as an intravenous bolus.

In **Chapter II** the estimation of the integrals of the unit dose impulse response function following oral drug administration (m-CBM/po system) is investigated. In this case the application of the linear trapezoidal rule may cause large errors. Therefore a new equation which may be regarded as a modification of the linear trapezoidal rule, using the ratio of the responses in the early phase as a measure of curvature, is

derived and its validity is demonstrated. Criteria for choosing the appropriate numerical integration method (linear and logarithmic trapezoidal rules, Simpson's rule, and the new equation) are evaluated. The combination of these algorithms, called 'mixed integration algorithm', is shown to be both simple and accurate, and has some advantages over the application of cubic polynomials and spline functions: it is more accurate in the calculation of the integral over the first interval, its application is less complex, and it does not give gross errors by spurious oscillations. Although the spline method performs well, the accuracy of the estimated integral over the first interval may be improved by coupling with the new equation.
In order to improve accuracy, sample points should be chosen in the early phase following administration of the unit dose, whereas in the terminal phase the sampling intervals may be enlarged.

In **Chapter III** the influence of data noise on the precision and accuracy of drug input calculated by numerical deconvolution is investigated both theoretically and experimentally. Applying the theory on error propagation, a set of equations describing the effect of data noise on the precision, expressed as the ratio of the coefficients of variation of the calculated input and that of the data points, is derived. The influence of data noise on the accuracy is determined as the systematic error caused by random errors in the data points. Experimentally the effect of data noise is studied in sets of simulation experiments in which random errors are added to the data.
From the results it can be concluded that the error sensitivity of the cumulative input calculated by numerical deconvolution is rather small if the unit dose is administered intravenously and the integrals are calculated using the mixed integration algorithm. The precision is of the same order of magnitude as the data noise, whereas the accuracy is hardly affected. The polynomial and spline functions are extremely sensitive to data noise, and should not be applied. It is shown that the constant input method is less sensitive to data noise if the unit dose is administered during the first interval. If the unit dose is administered orally, the influence of data noise is much larger, and the input cannot be calculated reliably in the presence of 10% data noise without the application of any method for reducing the influence of data noise.

In **Chapter IV** some methods for reducing the effects of data noise on the input calculated by numerical deconvolution are developed. A simple method for smoothing the calculated input, which improves both the precision and the accuracy of the calculated input drastically, is presented. If this principle is applied within the deconvolution procedure, the input profiles are damped effectively, and the error sensitivity is of the same order of magnitude as the data noise. The damping methods are shown to be superior to smoothing of the data points prior to deconvolution, since the latter procedure may introduce large systematic errors. The validity of two damping methods is demonstrated both theoretically and experimentally by simulations. In CBM/iv systems the accuracy and precision are satisfactory without damping, and may be improved by application of the damping procedure. The damping methods can be applied successfully for any CBM/po system, in particular in combination with the constant input method.

In **Chapter V** the algorithm errors due to the staircase approximation of various input functions are investigated. If the input profile obeys first-order kinetics, the calculated input is underestimated in CBM/iv systems. The maximum error is related to the ratio of the data points in

the early phase; the relative error in the calculated input does not exceed 4% if the response at the first sample point does not exceed the response at the second point. If the input rate rises during the first interval, or if the input starts after a lag-time, the input is over-estimated. In most cases the errors are rather small, and decrease if the number of data points increases. Applying the smoothing or damping methods the errors are increased. Therefore a gradually increasing damping factor Q_n is recommended, starting with $Q_1 = 0$, which provides accurate results. The sensitivity to data noise is comparable with a zero-order input.
In the calculation of in vivo drug release, i.e., if the unit dose is administered as an oral solution of the drug (CBM/po system), the algorithm errors due to the staircase approximation are larger than in CBM/iv systems. After application of the damping method the algorithm errors are decreased in most cases, thus increasing the accuracy. The double interval damping method appears to be less accurate. It is recommended to increase the damping factor gradually, starting with Q_1 is non-zero. The accuracy and precision of the calculated input are excellent, even at high input rates.

In **Chapter VI** two deconvolution algorithms, in which the convolution integral is approximated by the linear trapezoidal rule and Simpson's rule respectively, are derived. The accuracy and precision of the calculated input using the trapezoidal algorithm in CBM/iv systems is broadly comparable with the results using the staircase algorithm described earlier. However, the staircase algorithm has some distinct advantages, which are discussed. The Simpson algorithm cannot be applied to CBM/iv systems because the calculated input is unstable, even in the absence of data noise. After application of the damping procedure the Simpson algorithm appears to be accurate, but it does not perform as well as the staircase algorithm.
In CBM/po systems the precision of the trapezoidal algorithm is excellent, but the calculated input is less accurate. The accuracy may be improved by application of the Simpson algorithm, which is more sensitive to data noise. If the damping procedure is applied the results are comparable with the results obtained with the staircase algorithm. However, it can only be applied if the data points are equally spaced. Therefore the application of the staircase algorithm is recommended as the standard technique.

In **Chapter VII** two numerical deconvolution methods are compared for the case where data points are unequally spaced. It is shown that the best results with respect to accuracy are obtained by an interpolation method: first, a data set with a common time module is generated by interpolation, and then the deconvolution method is applied to the new data set. However, the interpolation method gives unreliable results in CBM/po systems if no damping is applied. Moreover, the efficacy of the damping procedure is considerably reduced.
The direct method, using only the original data points, requires formulae with time correction factors, and for each data point a different set of integrals of the unit dose impulse response function has to be calculated. Although the direct method may be inferior to the interpolation method with respect to accuracy of the calculated input, the direct method is more stable in the presence of data noise, and the precision can be improved significantly by application of the damping procedure. The introduction of systematic errors is reflected in the response values obtained on reconvolution.
Since data noise is inevitably present in real data, the direct method

with damping (Q_1 = 0.125, Q_n = 0.25 for n > 1) is recommended as a standard procedure. A check procedure using reconvolution, which allows a proper choice of the damping factors, is presented.

In **Chapter VIII** various methods for the assessment of drug input, including deconvolution techniques, statistical moments, and mass balance methods, are compared with respect to accuracy and precision of the calculated input. Two analytical deconvolution techniques and a numerical least-squares method are found to be more precise than the direct numerical deconvolution method with respect to the input rate, but in some cases the cumulative input profile is less accurate. The results of the Loo-Riegelman method are broadly comparable with the results of the numerical deconvolution method. It is recommended to calculate the mean input time (MIT) from input profiles obtained by deconvolution, since the calculation of statistical moments from data points is less accurate. It is concluded that the numerical deconvolution method with damping procedure performs satisfactorily when compared with the more complex techniques.

In **Chapter IX** the numerical deconvolution technique with staircase approximation is applied to real data. Several criteria for choosing the optimum numerical deconvolution technique are discussed. Three classes of criteria may be applied: the accuracy of the reconvolved response (reconvolution error), the smoothness of the calculated input profile, and the error sensitivity. Fine tuning of the damping factors can be performed by varying the damping factors, starting at the first data point, using the reconvolution error at the corresponding data points as a guide.
If the standard deviation of the data points is known, or can be estimated, the error sensitivity can be translated to a confidence interval, allowing a fair interpretation of the calculated input.
After deconvolution the input profile can be optimized by varying the input rate over each interval until the weighted sum of squares of the differences between the reconvolved and true response values (weighted least-squares, WLS) is reached. In order to avoid oscillations it is necessary to restrict the input rate to non-negative values. It is shown that this procedure may lead to smaller reconvolution errors, which seems to indicate a more accurate estimate of the true input profile. However, in most cases the input is less smooth when compared with the damping procedure, which seems to be less realistic.
In cases where a lag-time exists between the time of administration of the unit dose impulse input and its response, the normal numerical deconvolution technique cannot be applied, or may lead to large algorithm errors. It is shown that the input can be calculated by means of a lag-time correction. If the integrals of the unit dose impulse response function are obtained using the constant input method, the lag-time correction should be equal to, or a multiple of, the duration of the constant input, in order to avoid algorithm errors.
The numerical deconvolution technique is compared with the AUC ratio and statistical moments, which methods are alternatives for the assessment of the extent and rate of availability. It is shown that the choice of the data points for the assessment of the terminal elimination rate constant and the position of the last sample point strongly affect the AUC and mean residence time (MRT), whereas their effects on the AUC ratio and the mean input time (MIT) are much less pronounced. However, when compared with the deconvolution method, the error sensitivity of the AUC ratio and the mean input time and the effect of the position of the last point are significantly larger. Therefore it is concluded that

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the numerical deconvolution method is a more objective and precise technique for the assessment of the extent and rate of drug input.

In **Chapter X** the application of numerical deconvolution in biopharmaceutics is exemplified in a study on the in vitro and in vivo release of hexobarbital from hard gelatin capsules.
The in vitro dissolution rate of the hydrophobic drug hexobarbital from hard gelatin capsules was greatly enhanced by hydrophilization, i.e., by coating the powder surface with the hydrophilic polymer methylcellulose. A further increase of the dissolution rate was obtained by the addition of small amounts of disintegrants, and by particle size reduction before hydrophilization.
The effect of hydrophilization on the in vivo release rate was only slight when compared with the effect on the in vitro dissolution rate. Surprisingly, the extent of bioavailability (F is 0.84 relative to an aqueous solution of hexobarbital) and the release rate (mean input time MIT is 64 min) were slightly decreased when compared with the untreated drug (F is 0.89 and MIT is 53 min). The presence of disintegrant in the capsule formulation of the untreated hexobarbital appears to enhance the in vivo release rate to the same degree as hydrophilization. After particle size reduction and hydrophilization the highest in vivo release rate (MIT is 49 min) and extent of bioavailability (F is 0.95) were found.
It is concluded that there is no quantitative, and hardly a qualitative, correlation between the dissolution rate in vitro and in vivo of hexobarbital from hard gelatin capsules.

In Chapters XI to XIII some related subjects which arose during the development of the numerical deconvolution method are treated.
In **Chapter XI** a new criterion to choose between the linear and logarithmic trapezoidal rule, based on the sign of the second derivative (SSD), is proposed. The SSD criterion is part of the mixed integration algorithm described in Chapter II. It was found that a proper choice between the linear and logarithmic trapezoidal rule is particularly important in the application of Wagner's exact Loo-Riegelman equation. The method used by Wagner may lead to large errors in the calculated fraction absorbed. It is shown that Wagner's exact Loo-Riegelman equation is an improvement over the original Loo-Riegelman equation.

In **Chapter XII** it is shown that the mean input time (MIT), a general concept comprising the statistical moments mean absorption time (MAT) and mean dissolution time (MDT), can be obtained directly from input profiles. Equations for the calculation of the MIT from discrete data, from polynomials, and from polyexponential functions are presented. It is shown that truncation of the input at a certain time, e.g., the last data point, may be necessary in order to obtain accurate estimates of the MIT. When the input profile is gradually increasing or decreasing, an erroneous MIT may be calculated. It is recommended to choose the time point of truncation by visual inspection of the input profile, and after calculation of the MIT at each data point in the terminal phase, in order to obtain a reliable estimate of the mean input time.

In **Chapter XIII** a new method for calculating input rate constants from cumulative input profiles is derived. The commonly applied methods for calculating a first-order input rate constant, using linear regression techniques or graphical methods, require an estimate for the asymptotic value of the cumulative input at infinity. The use of an erroneous value of the asymptote may lead to large errors in the calculated value of the

rate constant. Therefore a new method for estimating the asymptotic value, where the optimum value is defined by a least-squares criterion, is proposed. Appropriate weighting factors, which are based on the variance of the data points of the cumulative input, are derived. The new procedure is discussed with respect to the basic conditions of linear regression analysis. The method can be applied to data pairs of any cumulative profile, e.g., absorption, in vivo release, or in vitro release.
The proposed method is applied to an example found in literature, where the usual method for the calculation of the input rate constant results in large errors, which are highly dependent on the number of data points used for the calculation. The authors of that paper wrongly attributed the errors to the Loo-Riegelman method. It is shown that the new method performs well; the calculated input rate constant and asymptotic value are close to their true value, and are almost independent of the number of data points used for the calculation.

Finally, the general conclusions of the investigations are translated to recommendations for the application of numerical deconvolution in the assessment of bioavailability and in vivo drug release.